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STRUCTURE AND PHYLOGENY OF PENTRAXINS

Insights into the Evolutionary Relationships and Functional Architecture of Pentraxins

[1]DIBYAJYOTI DWARI
[1]Fermentation Scientist,
[1]School of Biological Science,
[1]University of Portsmouth, Portsmouth, England, United Kingdom

Abstract:** The human plasma proteins c-reactive protein (CRP) and serum amyloid P component (SAP) are one of the considerable current interests as targets for drug interventions to target inflammatory and amyloid deposition diseases respectively. They are homologous members of a wider protein family, the pentraxin family, that share the same jelly-roll topology of subunit architecture and in many cases, a pentameric arrangement of subunits in their quaternary structure. Despite their similarity and likely evolution from a gene duplication event, the human proteins display distinct specificity for calcium-dependent binding of ligands that likely underline their role in biology. There is also considerable current speculation that different oligomeric states of c-reactive protein may exhibit different functionalities. The evolutionary history of the protein family is extended with related proteins present in most vertebrates and some invertebrates. The protein fold is also displayed in some plant proteins like concanavalin A and as a domain in bacterial toxins like botulinum toxin. The growing database of amino acid sequences arising from gene sequencing provides the results of an extensive mutagenesis experiment of nature, where the pentraxins have adapted to different physiological contexts. A study of the properties of these molecules may provide insights into the behavior of human proteins. For example, it is known that the female hamster pentraxin recognizes phosphocholine and amyloid fibers, specific ligands for the different human proteins. The zebrafish protein assembles as trimers while the protein from the horse-shoe crab forms heptamers. With regards to that human pentraxins are pentameric. Therefore, studies regarding these proteins can give a fundamental understanding about the structure and function of these proteins. Structural variation is also found in the case of different types of species in evolution due to various biological factors. Can these molecules inform us about the hypothesized instability of human CRP? The main objective of this study is to find out more information about the structure and behavior of these proteins.

***Keywords:-** Human plasma proteins, C-reactive protein (CRP), Serum amyloid P component (SAP), Drug interventions, Inflammatory diseases, Amyloid deposition diseases, Pentraxin family, Jelly-roll topology, Subunit architecture, Pentameric arrangement, Quaternary structure, Calcium-dependent binding, Ligands, Evolution, Gene duplication, Oligomeric states, Vertebrates, Invertebrates, Concanavalin A, Bacterial toxins, Botulinum toxin, Amino acid sequences, Gene sequencing, Mutagenesis, Physiological contexts, Female hamster pentraxin, Phosphocholine, Amyloid fibers, Zebrafish protein, Trimer, Horse-shoe crab protein, Heptamer, Pentameric human pentraxins, Structural variation, Biological factors, Instability of human CRP, Protein structure, Protein behavior*

1. INTRODUCTION

The development of pentraxins in the horseshoe crab was very early in its evolution. It has been estimated that these proteins have existed for around 250 to 300 million years [1]. Despite this, detailed investigation of their functional characteristics remained unclear. The revelation of C-reactive protein (CRP) in case of man was accomplished fortunately in examination of the plasma of an infected person who was suffering with extreme *pneumoniae* species [2]. This CRP showed up in the blood when the diseased individual was fundamentally sick. It was not recognizable before the contamination and after the disease cured. Therefore, the clinical examiners of Rockefeller University proceeded to biochemically describe this type of protein. CRP was available in exceptionally high focus in intense stage of serum, and it would actuate condensation of pneumococcal cell divider removes yet just within the sight of calcium [1,2].

The pentraxins are a group of proteins having a fivefold shape that is rather unusual. They work as pattern identification molecules, identifying external antigens and changed own-antigens and tagging them to trigger natural defense system. This trait is shared by inborn molecules that existed before immunoglobulins were developed [3]. Pentraxins also stimulate immunological feedback by interconnecting along with defense mechanism and cell receptors. The pentraxins along with receptors for the Fc portion of antibodies were thought to have occurred before the creation of antibodies inside the body.

In the case of humans CRP and serum amyloid P component (SAP) are analogous proteins in other species which are considered as members of plasma proteins. They're made up of five non-covalently linked protomers which are organized in a disc-type structure with cyclic pentameric arrangement on a symmetrical plane [4]. Each site possesses a calcium-dependent binding region that facilitates the binding of ligand conforms that, these proteins' carries a well-documented functional characteristic. Even though their exact activities in humans are not clear, gene experiments in case of mice show that they can play a role in natural immunity [3,4].

CRP has a molecular mass of 115,135 Daltons, while SAP has a molecular mass of 127,310. CRP & SAP are made up of fivefold packed subunits that are organized in a plane symmetrical structure [5]. The molecule looked to be a doughnut-shaped ring, according to electron microscopy. Although it was previously thought that CRP was made up of a single pentameric structure and SAP was made up of decamers, it was subsequently shown that the SAP component, flows in the plasma as a single fivefold symmetry like CRP molecule [6]. CRP's fivefold shape provides it with a superior level of firmness and opposition to biocatalytic assault. CRP and SAP have a lot of structural and biological similarities. Both are a calcium-dependent cyclic pentamers that bind to ligands [7].SAP component having a crystallographic structure showed the molecule's five-fold symmetry and the calcium-determined attachment site is for the specific binding of 4,6-cyclic pyruvate acetal of -D galactose and phosphoethanolamine. The first crystallographic model conforms this" [8]. A preserved -sandwich fold having dual countering -sheets makes up each pentraxin protomer. The phosphocholine (PC) binding site was postulated to have a aquaphobic unit on each side of the subunit, with identities along with variations between the pentraxin attachment process. Just after that, a crystallographic structure of the collaboration between CRP along with PC ligand was developed [8].

The pentraxins are a protein family that includes CRP and SAP component Which perhaps a novel discovered identical molecule generated from endothelial cells named as Pentraxin 3(PTX3) [9]. They are distinguished by their unique composition: five or ten homologous subunits having superior amounts of similarity among proteins and the coding genomes. They have calcium-dependent binding to various ligands like phosphate esters, polysaccharides, and polyelectrolytes. In general, enhanced biogenesis of this protein produced by inflammatory and necrosis products [10]. The group of protein was discovered in the limulus as well as in every vertebrate variety investigated so far, confirms that they have genuine survival significance. The proteins have been thoroughly studied at the human gene and genome levels by referring to several representative lower species.

In the 1990s, the group of long pentraxin like PTX3 were discovered as triggering genes or molecules expressed organs such as neurons and spermatozoa [11]. Long pentraxins are different as of short PTX in genome structure, cellular resource, Cellular stimulation, and ligand-specificity capabilities. PTX3 in general functions as a soluble molecule for the sample recognition of receptor, serving a nonreduced job in inborn defense in contrast to certain foreign elements [12]. This protein also plays a nonfunctional character in female fertilization owing to its underlying attachment in the extracellular matrix space. It was also shown as an active function in infection by serving as a feedback process that inhibits leukocyte [11,12].

The group of long pentraxins were discovered after PTX3 was identified in guinea pig apex-in known as neuronal pentraxin named as NP or NPTX1. Similarly, NP2, also known as Neuronal pentraxin-2(NPTX2), the sole representative with a transmembrane domain [13]. NPTX2 has been linked to endless neural flexibility and dopamine induced neuronal cell death, whereas NPTX1 has been linked to amyloid-induced neuronal cell death [14]. The structural domain of this protein was also discovered in multifaced proteins, like the excess cellular space Polydor [where the N-terminal element of A face contains two hyalin duplication units]. There were also a few linkages of G-protein–coupled receptors (GPRs), specifically GPR144, GPR112, and GPR126[15,16].

Pentraxins (PTXs) is a conserved protein superfamily that is part of the immune system and has large effectiveness in the immune mechanism. The proteins are thought to be practical progenitors of antibodies which are divided into two types: Long type PTX and short type PTX. Through the investigation, it was confirmed that a PTX like domain with a C-terminal is strongly like the short pentraxins of humans pentraxin domain. Similarly, a full N-terminal domain, which is characteristic of having long pentraxins, is implicated in the immune comeback of *Ciona robusta* in living body [17]. The strong link between PTX-like and mammalian CRP was confirmed by protein domain analysis, 3D modeling, and a phylogenetic tree, conforms that *C. robusta* PTX and mammalian CRP have a common progenitor in the diverse group of chordate lineages [16,17].Two groups required gene of interest was separated from human monocytes, and epithelial cells activated by IL-1 which was described as a cloning genetic factor producing a domain including a carboxy-terminal of amino acid sequence similar to human short pentraxin in 1992-1993 [18]. One group called the gene 'TNF-stimulated gene (TSG)-14,' while the other called it 'pentraxin 3 (PTX3),' and was found in cytokine-stimulated mice monocytes. Specific amino terminal having 181 residues were found to have no significant similarity with pentraxins and its groups [19].

The PTX3 gene was investigated to have three exons, from which the third one is roughly equivalent to the CRP and SAP second one [20]. Tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-6 associated with glucocorticoids promote its regulation, which is comparable to that of PTX genes. TSG14 along withPTX3 emerges for a short period in blood serum after inducing a critical point reaction with endotoxin and demonstrates immunologic reaction inside human body, although immunohistochemistry investigations show that, the principal site for CRP and SAP synthesis is outside the liver[20,21].

This origin of gene was encoded as 381 amino acid, 42 kDa protein (about double the size of a CRP or SAP domain) with just 200 carboxy-terminal residues in common with pentraxins and a hydrophobic signal peptide sequence. The pentraxins have been linked to a variety of biological roles, the most consistent and well-studied of which include triggering of the complement system and phagocytic cells, implying a activity for these proteins in innate immunity [22]. In humans and numerous typical lower species, the proteins have been well investigated at the genomic and proteomic levels. Nonetheless, the major activities of this protein family appear to be unknown. The protein fold in diverse group of organisms has not been clearly understood to know the exact behavior of this type of proteins [23].

2. METHODOLOGY

Various Bioinformatics tools are followed to determine the structure and phylogenic data for the experiment. Selected group of genome information of targeted sequence were obtained from the Swiss-port database(www.Ebi.ac.UK/Swissprot/), and Expasy (https://www.expasy.org/). Sequences were aligned by a bioinformatics Search tool system called basic local alignment (BLAST). BLAST is a tool of bioinformatics that performs the equality search between the pairs of sequences for determining a local similarity. The most essential (and misused) of the three terms is “Sequence homology”. When we state that sequence A and sequence B have high homology, we're implying two things: first, that sequences A and B appear very similar, and second, that all their ancestors looked very similar, all the way back to a common ancestor [24]. The first of these claims can be easily verified, but the second is usually questioned. Although the comparison of two sequences is frequently described as a percentage sequence homology, this is generally erroneous because of result show’s identity and/or resemblance rather than an evolutionary link.

The Clustal-W algorithm was used to align the exon containing sequences or amino acid sequences of pentraxins, which were subsequently uploaded into BLAST database for genetics analysis for the creation of phylogenetic trees, two different algorithms were used: the “maximum-parsimony” technique and the neighbor-joining technique [25]. The following distances were used to create NJ trees: the uncorrected percentage of amino acid difference denoted as (p) and the Poisson-corrected proportion of variations between the sequences. Phylogenetic tree findings are displayed in ***Fig.2***, which depicts the family for a selection of both pentraxins. When positioning coding amino acid sequences, similar findings were achieved. The Expasy (https://www.expasy.org/) and NCBI (http://ncbi.nlm.nih.gov) records have being used to obtain data for orientation of the PTX3 and PTX4 genome sequences, in addition to know their genetic placement. There are a series of bioinformatics tools used to get the results.

Various 3D structures of the protein molecules were predicted by the computational tool AlphaFold. This is the tool taken for predicting the structure because AlphaFold structures far outperformed other approaches in terms of accuracy [26]. “The median backbone accuracy for AlphaFold structures was 0.96 r.m.s.d.95 (C root-mean-square deviation at 95 % residue coverage) (95 % confidence interval = 0.85–1.16), while the median backbone accuracy for the next-best method was 2.8 r.m.s.d.95 (95 percent confidence interval = 2.7–4.0)” [27]. “When the sequence structure backbone is extremely accurate, AlphaFold can construct highly accurate side chains, and it outperforms template-based techniques even when powerful templates are available. Compared to the best alternative method's 3.5 r.m.s.d.95 (95 % confidence interval = 3.1–4.2), AlphaFold's all-atom accuracy was 1.5 r.m.s.d.95 (95% confidence interval = 1.2–1.6)” [26,27].

Another bioinformatics tool was used for visualization of 3D structure of pentraxin domains. For observing the biomolecule structure and its subunits in the native state like proteins, DNAs, tiny particles, electron concentrations, and paths, PyMOL, a multi-platform molecular graphics application, has been extensively used predicting different parameters [28]. It can also create videos, alter molecules, and use ray tracing. The goal of this Python-based program's development was to improve its functionality and make PyMOL's drug creation process easier. This software presents an extensive illustration on various molecular modelling modules in PyMOL, which covering all aspect of visualization and analysis enhancement, protein–ligand modelling, molecular simulations, Side chain identification, calculating the distance between the atoms in order to gain an understanding of useful structural tools and their useful applications in PyMOL [29].

Another tool called as PDB SUM(http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html) have been used to generate topology diagrams. Generally, this tool is used to observe details about the protein structure [30]. The tool shows necessary parameters like metal ions, ligand binding site, Helices, different folds, hairpin loops, and protein chains. This helps to understand how the group of proteins fold to give a specific type of structure and how they are different from each other.

3. RESULTS

3.1 Phylogeny of Pentraxins

Phylogenetic analysis was performed to find sustained sequences among mammals, lesser vertebrates, nematodes and arthropods to better study the cross relationship between known as the pentraxins (Short and Long) and their ancestor. Using large systematic BLAST searches, all accessible homologous sequences of desired pentraxins were collected from multiple genome sequence databases. Orthologous molecules of the short PTX like CRP and SAP, and the long PTX like PTX3. The Neuronal pentraxin (NP1, NP2) have been discovered in mammalian species as well as below vertebrates like zebrafish, and frog (*Xenopus*) [31].

In case of hamsters the female protein is represented as the short pentraxin shows similarity to SAP. The female hamster expresses high frequency of pentraxin which is variably controlled during pregnancy in diverse hamster species. In the case of *L. polyphemus*, homologous of the short PTX, in addition to the long-sighted pentraxin (PTX), was discovered in arthropods. A shorter pentraxin termed Mptx (NM 001037642) has been identified in the rat; Mptx is a group of pentraxin whose activity is suppressed by different folds. This group is distinct from other short PTX, as per research and has less homology in the mouse and human.

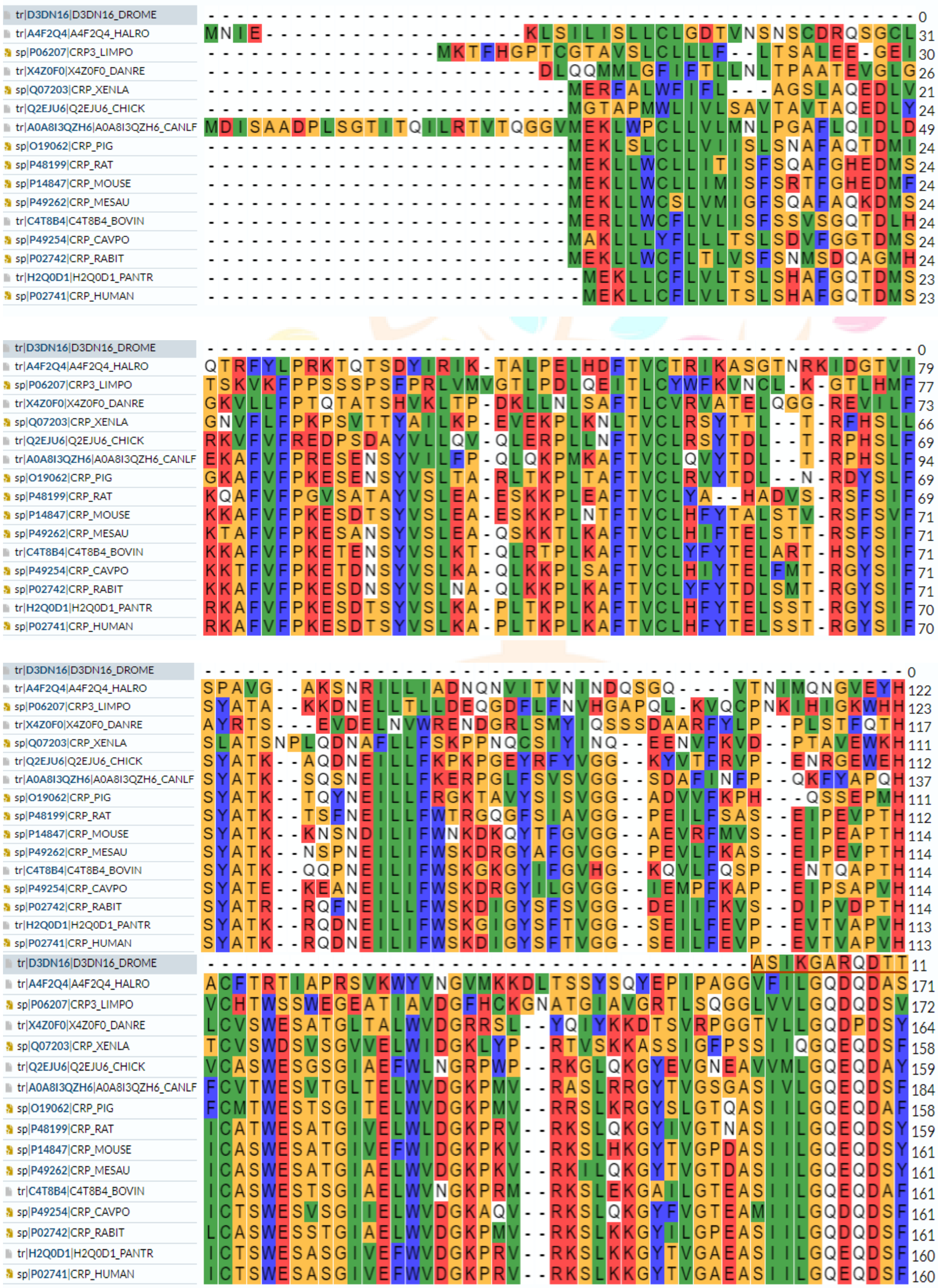
Phylogenetic tree was constructed to gather information about strategic evolutionary relationship and conservation of the protein folds for the possible mutations. The changes were observed by taking into consideration of following species.

The species analysed were: *Homo sapiens* (human); *Rattus norvegicus* (rat); *Takifugu rubripes* (pufferfish); Oryctolagus cuniculus(rabbit); *Drosophila melanogaster*(fruit fly); *Mus musculus* (mouse); *Cricetulus migratorius* (grey hamster); *Cavia porcellus* (guinea pig); *Xenopus laevis* and *tropicalis* (African clawed toad); *Danio rerio* (zebrafish); *Gallus gallus* (chicken);

Mesocricetus auratus(golden hamster); *L. polyphemus* (horseshoe crab);*Canis familiaris*(dog); *Sus scrofa* (pig); *Monodelphis domestica* (opossum); and *Anopheles aegypti* (mosquito).

3.1.1 Systematic BLAST search

To investigate more about the protein presence in distinct species a BLAST search was conducted. For doing that different group of species like mammals, arthropods, birds, amphibians, reptiles, and flies' species were taken for analysis. The species from all over evolution were investigated to understand the sequence identity and changes during the evolution.



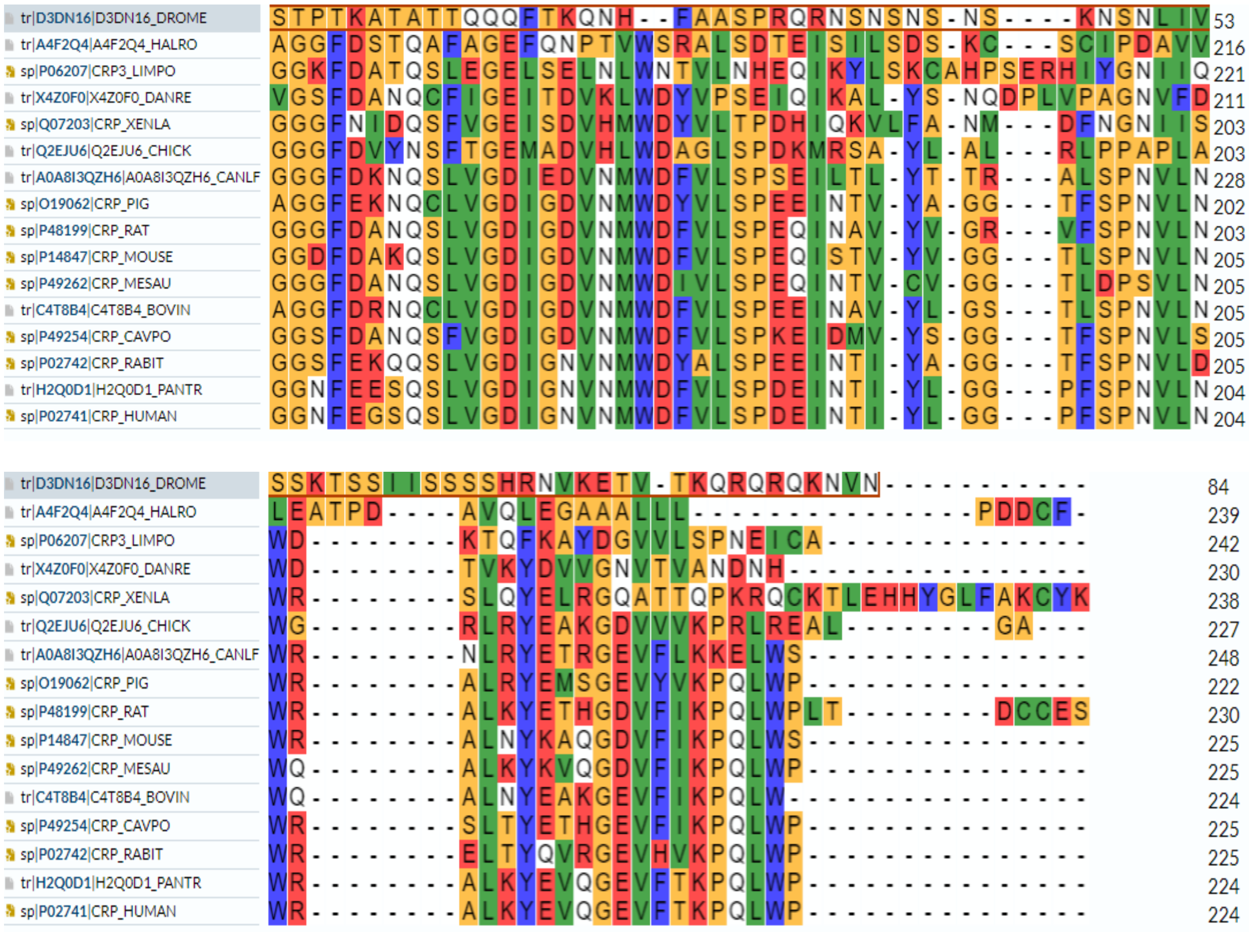


***Fig.1** Multiple Sequence alignment of the short pentraxin CRP found in selected species from the uniport database showing the similarity score of different amino acids and the possible gap penalties between them. Differences between sequences are further analysed in result section. Highly conserved region is marked as (Red), Gene annotations (Yellow), hydrophobic, non-aromatic regions(green) and low amount of conversion rate are denotated as (Blue)*

3.1.2 Phylogenetic tree Analysis

The group of short PTX like CRP and SAP are diverged, as of the parent regenerator of all PTXs. This type of pentraxin was identified in diverse groups of chordates (mammals), arthropods (Limulus), and Amphibians like Xenopus denoted as long pentraxin (XL-PXN1), which was represented as the first cluster in phylogenetic tree (Fig. 2). Limulus pentraxins appeared on the series of branches prior to mammalian short PTX, constituting a distinct taxonomy. The pentraxin were named according to their presence in the common ancestor sequence.

The second cluster comprises of Neuronal pentraxin (NPTX) represented like a subgroup of long PTX discovered in both higher animals and in below type craniates (Fig. 2). By observing the branch size of NPTXs, NPR is the oldest pentraxin which was different from a parent regenerator of NPTX [32]. Similarly, the NP2 and NP1 were observed in the case of human, murine, and rat homologous which is not identified till now. It was predicted that that NP2 was homologous due to sequence homology, although the chromosomal localizationism was limited to guinea pig not represented as the group for NP2.

Only PTX3 is found in the third cluster, and its sequence has been found in mammals, also in the case of flora (G. gallus) (Fig. 2). This was also found in the most common oldest craniate, *T. rubripes* (pufferfish). Thus, the long pentraxin named PTX3 descended drastically from the parent regenerator pentraxin sequence during the divergence of vertebrates, when pentraxins were still evolving. But almost it gives a clear-cut idea about how the sequence was conserved in the case of some higher species. This might be due to cross mutation and the environmental changes impacting the sudden change in common ancestor.

Finally, the protein of *D. melanogaster* (Fruit fly) was investigated. The pentraxin protein is composed of 558 amino acids whose biological purpose is not known, and *A. gambiae* protein comprise the last cluster (Fig.2). Pentraxins have a common ancestor, and B6 protein is one of them. A BLAST search of the B6 gene sequence against the human database revealed that there is no putative human ortholog of B6.

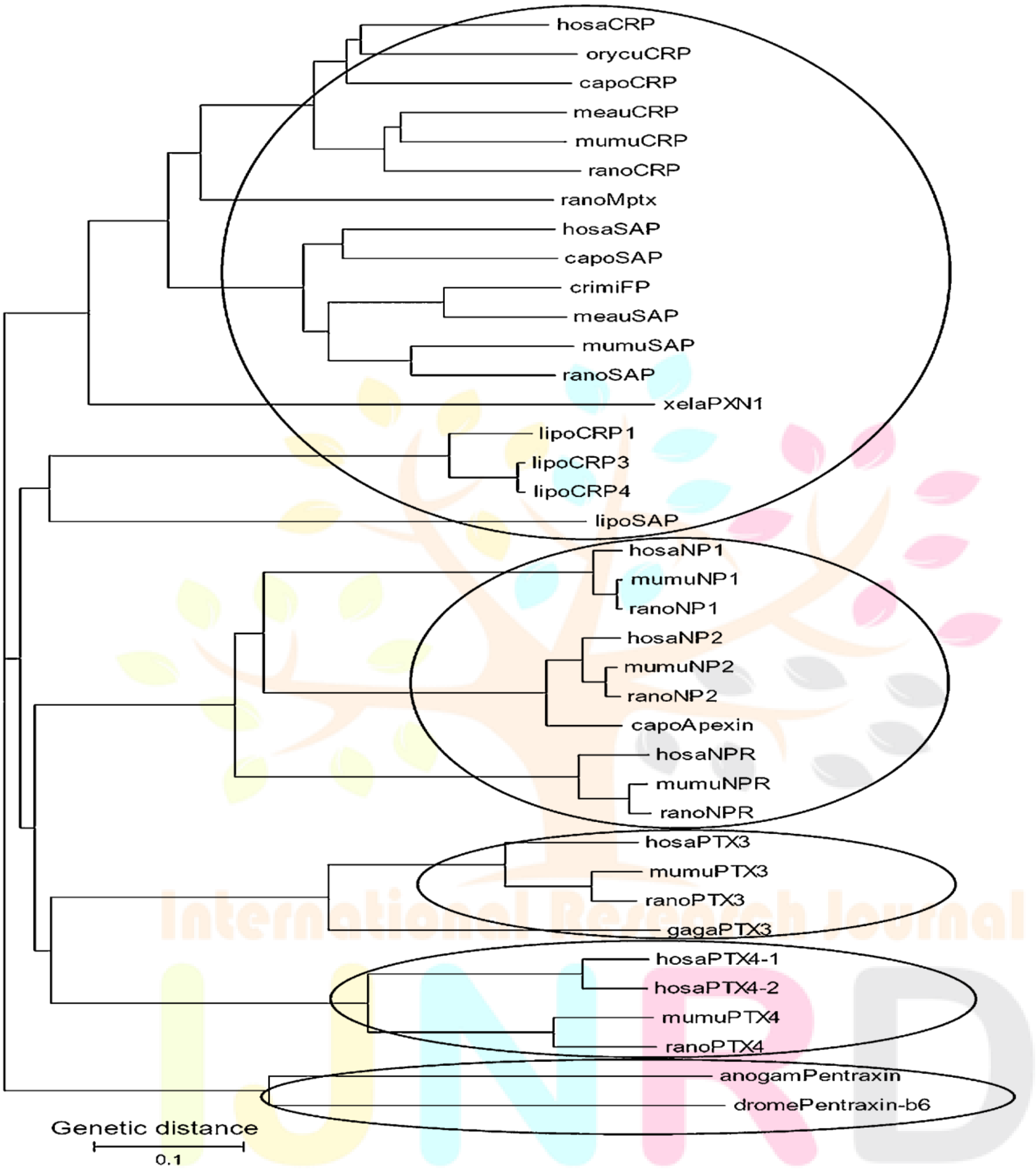


Fig.2 *Phylogenetic tree was constructed by taking PTX group. These groups of long and short pentraxins were represented along the name in different clusters. There are 5 clusters in this tree. NP1 and NP2 were represented as the subgroup of Long PTX. PTX4 is the new group of long pentraxins shown in cluster-4 having high identity towards the common ancestor.*

3.1.3 A novel protein pentraxin: PTx_4

Researchers used the apex in search of amino acid sequence for unknown PTX in biological databases. This is an effort to discover novel PTX domain-including proteins, particularly the mouse model as well as human equivalent of guinea pig PTXs. This specific sequence of nucleotide was used as the initiator for searches for related but unidentified sequences in the mouse, zebrafish, opossum, xenopus, rat, human, cow, dog, and pufferfish genome databases. This search indicated the discovery of a novel long pentraxin, which we have named PTX4 and contains about 470 amino acids and is distinct from apexin. Rat, mice, and human PTX4 exhibit around 30% pentraxin domain identity and 10% N-terminal identity with other long pentraxins. Similarity score varies from 35–49% including higher vertebrates which is 51–88% in case of animals in Custal W sequence placement of PTX4 sequences [31,32].

A well-known gene containing 3 exons was found when the genomic orientation and position of the PTX4 gene in human, murine, rat, and opossum was compared. The exons are practically all the same length, and each intron has 39 splice acceptor sites and 59 donor sites that are easily identifiable.

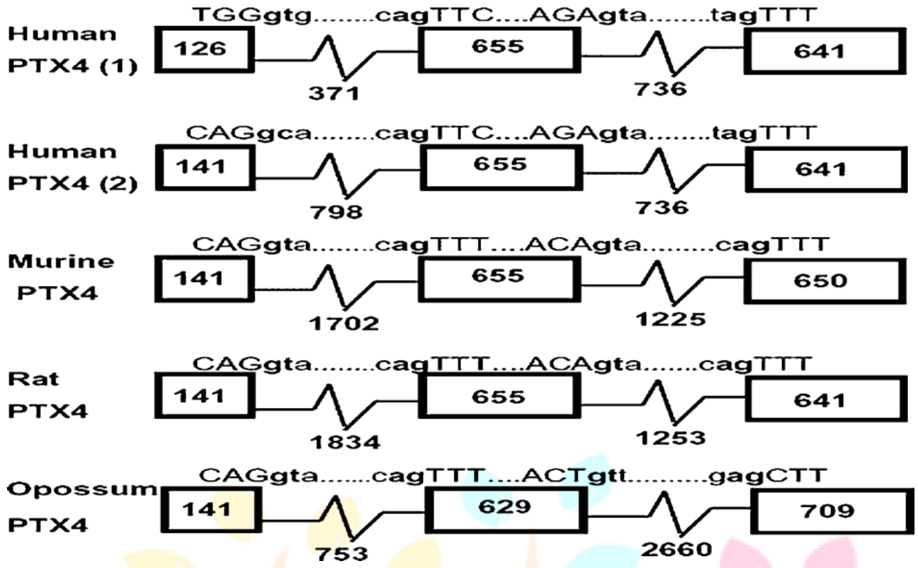


Fig.3 *PTX4 gene analysis. Comparing the genomic organization of the PTX4 genes in humans, mice, rats, and opossums. Boxes stand in for exons. Intron sizes are listed beneath the introns, while exon sizes are specified within the boxes. Each splice site's three surrounding nucleotide residues are displayed; coding residues are denoted by capital letters.*

3.2 Structure of pentraxins

Structural investigations of pentraxins revealed that they are Five identical, non-covalently coupled, and no glycosylated subunits that make up native pentameric CRP. These subunits are organized in a cyclic disc-like arrangement around a central pore. For every single fold has a molecular mass of roughly 23 kDa [33]. They are bent into 2 antiparallel sheets as well as a compressed roll shape. Between these two sheets lies a hydrophobic core, having single end of the center exposed to solvable element. Generally, there are five folded subunits form a ring having a diameter of 20A and 35A depth in the center, which contains a positive charge cluster Ionic interactions stabilize the decamer, allowing access to the incurvate facing side of each pentamer [34].

Both proteins CRP and SAP are made up of five tightly packed subunits (protomers) that are arranged in a planar symmetry. The molecule was discovered to have a doughnut-shaped ring using electron microscopy. It was thought that CRP structure comprised of a single pentamer, and SAP component was made up of decamers. But after the later experiments it was shown that SAP moves in the plasma as CRP following a single pentameric structure [35]. CRP's fivefold shape gives it an elevated level of stability to two Inflammation caused by enzymatic attacks. CRP and SAP component have many biochemical similarities. They are both calcium-dependent cyclic pentamers showing different ligand specificity. They are 51 % identical in amino acid sequence and have highly comparable structures SAP. CRP is comprised of a glycosylated subunit and carbohydrate part [34,35].

The first crystallographic structure of PTX revealed SAP is pentameric and it has the calcium-dependent binding site. The site has ligand specificity towards the 4,6-cyclic pyruvate acetal of D-galactose and phosphoethanolamine. When the first crystallographic model has being published, the structure of CRP was clearly defined [36]. A preserved overlapping fold with both site opposing face makes up each pentraxin protomer. The phosphocholine (PC) binding site was postulated to be a hydrophobic core site for the protomer, with identity and variations noted between short PTXs binding sites. A crystallographic str ucture prediction between CRP and the PC ligand was done after that.

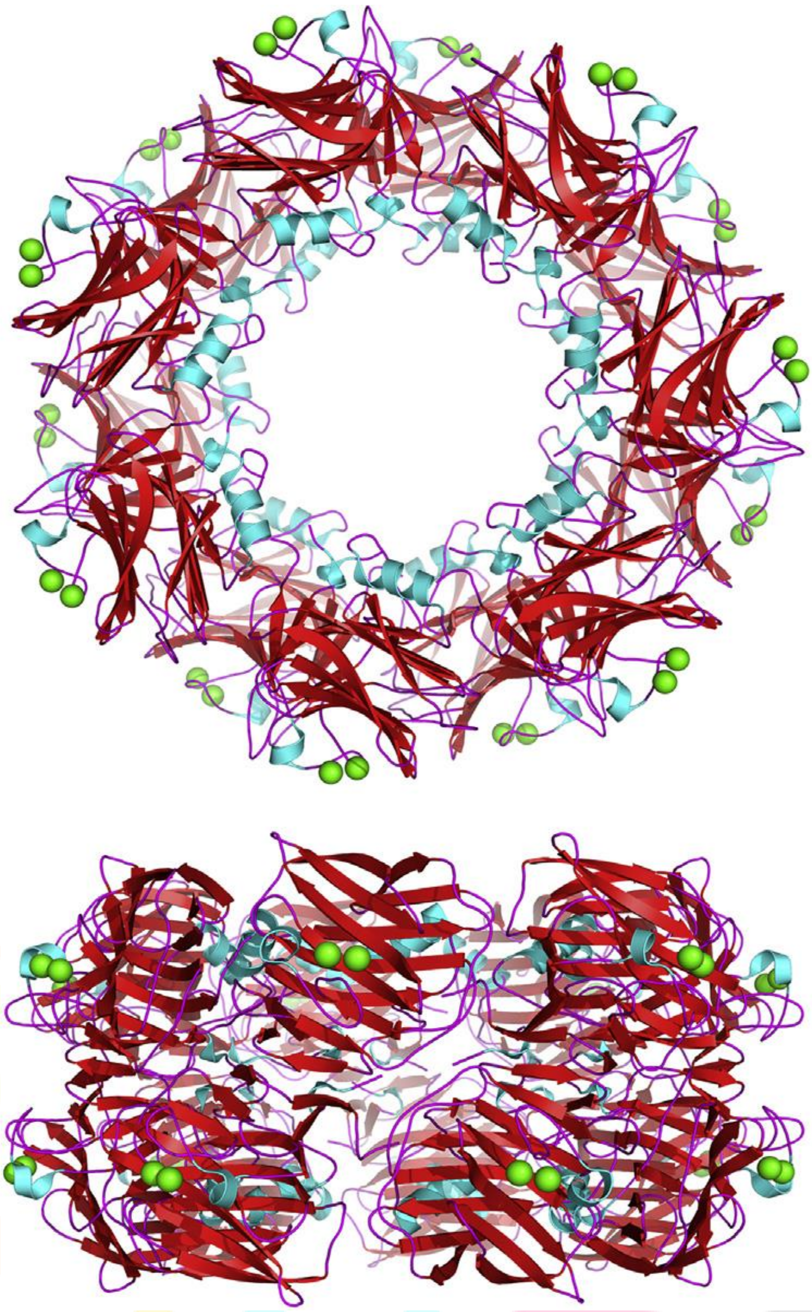


Fig.4 *Seven folded protomers combines to form heptameric Horseshoe crab SAP component shown in terms of ribbon diagram as vertical and upright to the core and cyclical towards the axis of the core band shape. Purple is loop and red is the β strand. The Ca^{+2} ions are represented in green colour are associated with the perimeter of pentraxin with two number of Ca^{+2} per protomer.*

The "Jelly Roll" Fold

The pentameric structure of CRP follows the "jelly Roll/ Swiss Roll fold" arrangement. Each protomer of the pentamer is composed of this roll structure. This roll is formed by the arrangement of different beta strands and sheets provides support to the parent strand. So, each sheet of the protein fold acts as a supporting structure for the monomer. This results the tightly packed monomeric structure as shown in ***Fig.5***.

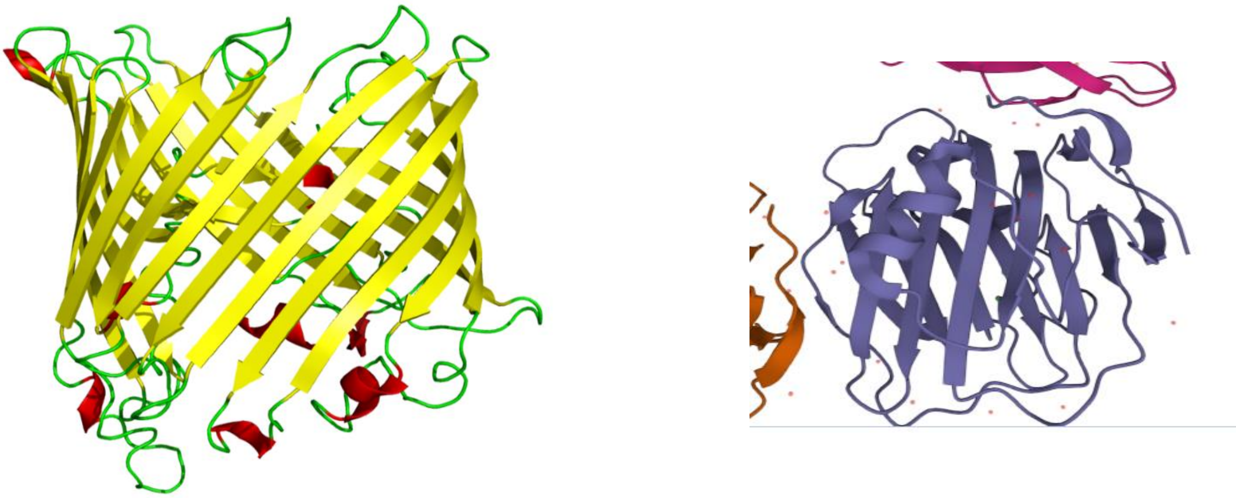


Fig.5 *Each monomer follows the "jelly roll" topology. The monomeric structure formation is quite similar with Swiss roll cake. By the folding of beta strands, it generates the packed protomers.*

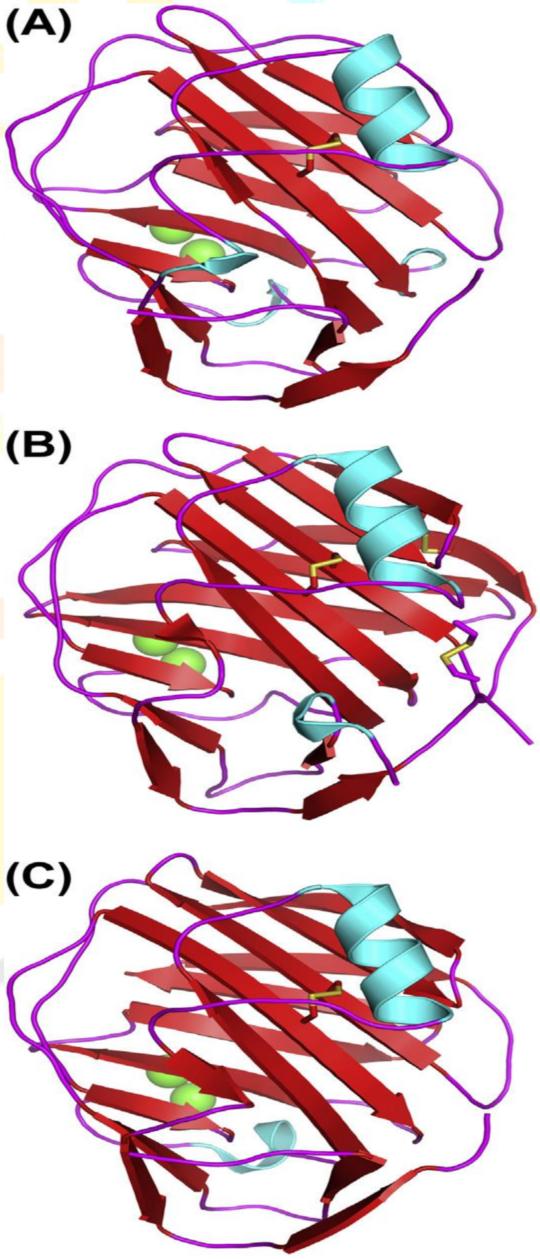


Fig.6 *The folded structure of different sort pentraxins shows less structure uniqueness. (A) Human CRP (B) Horseshoe crab SAP (C) Human SAP component as represented in this figure. The centre is represented as two folds of antiparallel β-strands (red) where "Jelly Roll" topology is same in all lectins-based fold protein superfamily.*

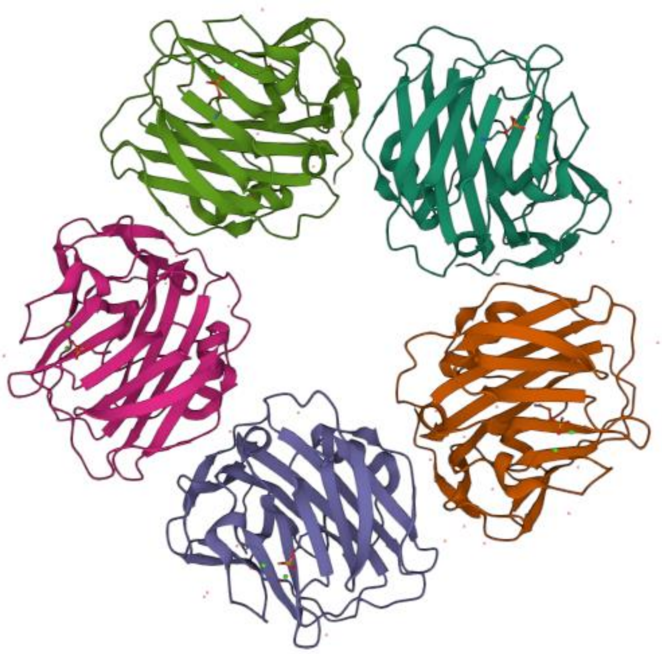


Fig.7 *The structure of CRP bound to phosphoethanolamine. The binding of ligands is represented with sticks of different colors. In every face it contains two numberCa*$^{+2}$ *ions.*

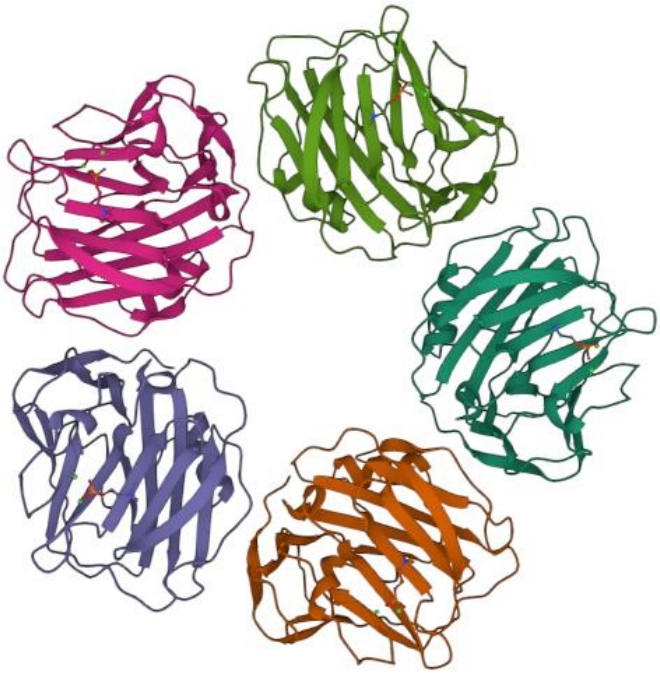


Fig.8 *Native structure of CRP molecule showing pentameric structure in a planner symmetry. Each pentameric structure contains five protomers which forms the pentameric structure.*

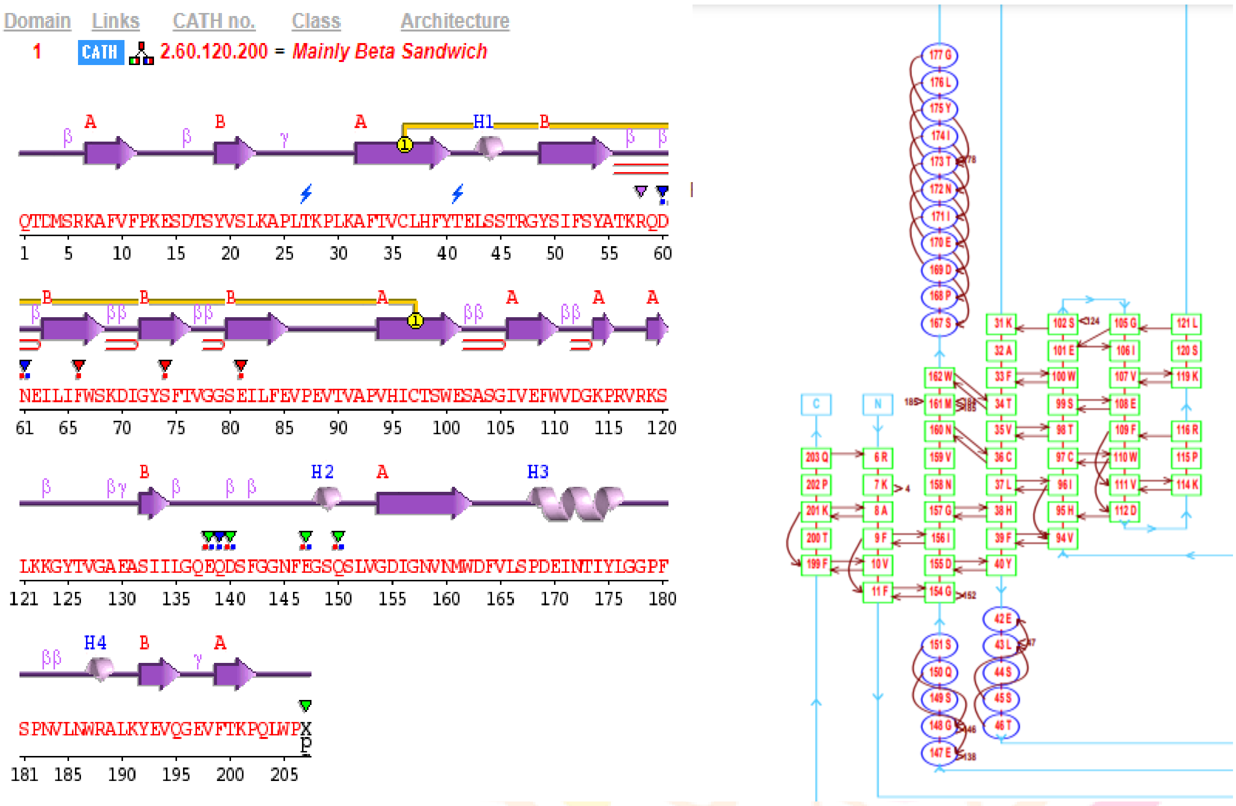


Fig.9 *Topological representation of pentameric CRP showing different structural arrangement of the molecule. The symbols represented different structural components are given below. This structure was taken from PDB sum database having PDB Id:1b09*

Helices like H1, H2, H3, H4

A B The sheets,

The beta turn

The gamma turns.

Beta hairpin loops

Di-sulphide bridges

Variant residues

Ligand Site

Metal site

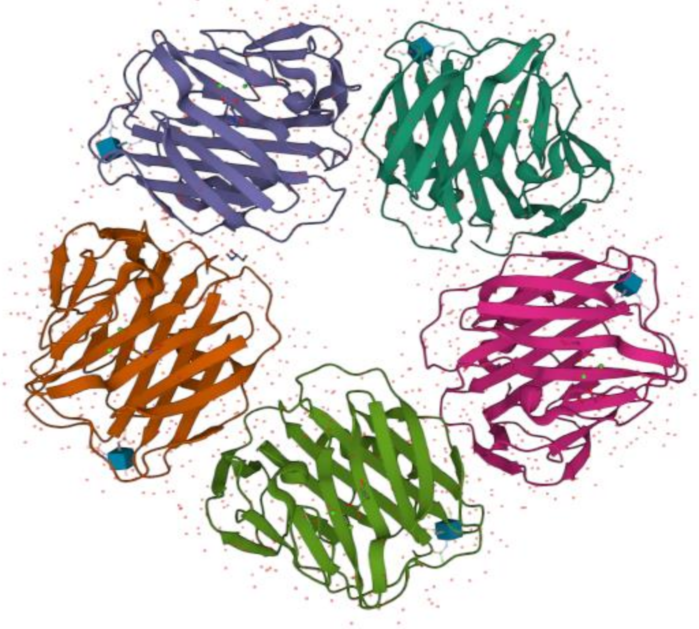


Fig.10 *Structure of serum amyloid P component binding to N-acetyl-D-Proline. The blue colours are represented as the N-acetyl-D-Proline in each protomer.*

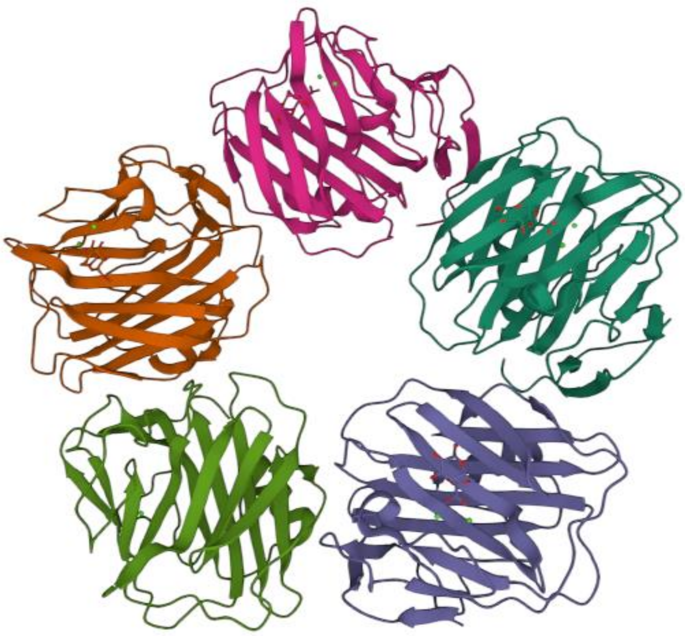


Fig.11 Native pentameric structure of Human Serum Amyloid P component showing five protomers and subunit.

3.2.1 Ligand Specificity

The interface of CRP ligands which expressed in case of bacteria and tissue necrosis was the target primary research on CRP. CRP was first discovered and called because of its attachment with C-polysaccharide, which is a key element of *S. pneumoniae* cell structure [37]. The attachment of CRP to the specific polyose was discovered previously on to be mediated by PC molecules, which are present in terms of teichoic acid and lipoteichoic acid as a crucial element of cell wall.
Fig.12 showing that Calcium was required for PC binding.

PC was found on several pathogenic organisms, and CRP has affinity towards it. PC is also known as the group of phosphatidylcholines, as a key element of animal's cell membranes. Usual nourishing cells do not have this PC class unit, exposed to outer region. Several type of foreign materials that might trigger the defence system can be present which are released by injured cells [38]. In some cases, these are autoantibody targeting material to the abnormal connective tissue. High-tittered antibodies against DNA And RNA complexes was targeted by systemic lupus erythematosus (SLE). The binding of short PTXs to these nuclear antigens, causing them to be cleared and processed differently. CRP attaches to small nuclear ribonucleoproteins (snRNPs) in cells, while SAP component affinity towards native DNA [37,38].

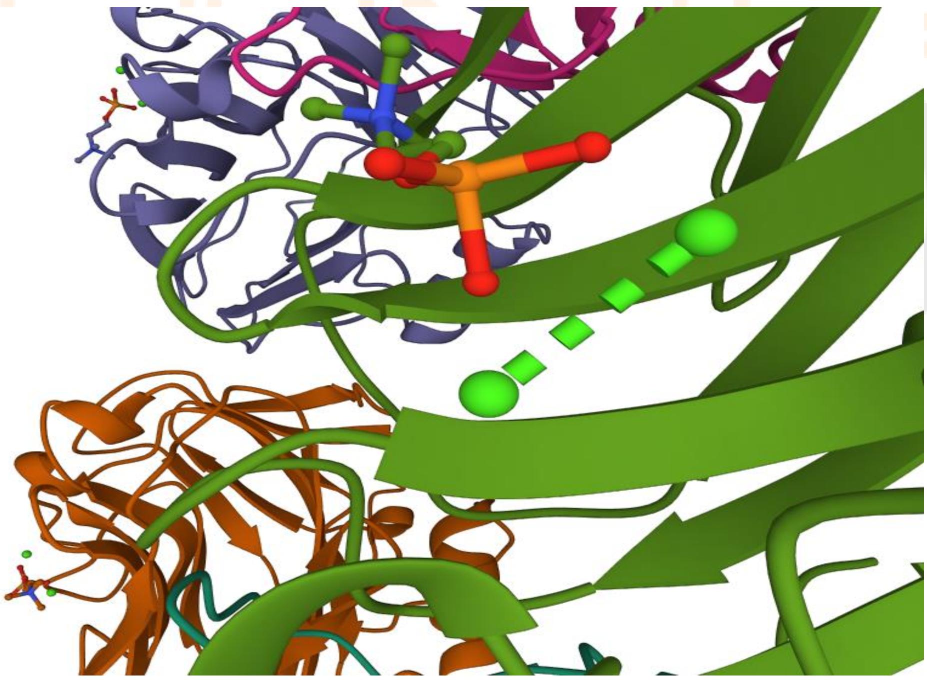


Fig.12 Side Face of CRP, each face comprises of 2 calcium ions for binding to presented in green colour.

Properties	*Human Serum amyloid P component*	*Human C-reactive protein*
Ca^{+2} dependent binding	Yes	Yes

Fc region Binding Affinity	Yes	Yes
Ligand Specificity	Amyloid fibrils Heparin Phosphoethanolamine Apoptotic cells DNA, chromatin	Oxidized LDL Phosphocholine snRNP (Sn, RNP) Histones Apoptotic cells
Site for synthesis	Liver	Liver
Glycosylation	Yes	No
Inducers	Constitutive	IL-6(Acuate phage)
Chromosome location	1q23.2	1q23.2
Structure	204 amino acids Cyclic pentamer 127,310Da Each subunit 25,462Da	206 amino acids Cyclic pentamer 115,135Da Each subunit 23,027Da

Table No.1 *Shows the detailed comparison between CRP and SAP Component*

It has been experimented that CRP binds to polycations. In contrast to template ligands such as PC, CRP binding to polycations is suppressed by calcium rather than PC. CRP binding to polycations has yet to be assigned a physiological function. Multivalent binding to each type of ligand, on the other hand, activates complement via C1q. SAP possesses identical attachment sites on the B site of 3D structure, but because to variations in the hydrophobic pocket, it fails to attach to PC [37,38] . However, the interaction for human SAP is significantly higher than mouse SAP. This discrepancy could make studying the involvement of pentraxins in mouse models more difficult.

3.2.2 Other structural analysis

Before the X-ray crystallography and comparison data were published, various relevant structural and biochemical role of the pentraxins studies were performed. The partial location of the Ca^{+2} binding site(s) short PTXs were more investigated by synthetic peptides, autoradiography, monoclonal antibodies, circular dichroism (CD) measurements and enzymatic digestion were used in studies [39]. In native state the CRP is not solvent-friendly, according to the three-dimensional prototype of the PTX structures developed from X-ray analysis. consequently, a relative shift might be useful to reveal this possible site of interaction. A single unknown peptide, but not the full CRP molecule, can prevent cell adhesion supports the fact. Structural investigations of several species are given below.

Limulus: - As previously reported in prior investigations, the horseshoe crab hemolymph lectin was known as 'limulin' which is represented as the CRP for this species (but it might very well be termed its SAP). Limulin is similar to CRP in terms of proteomic and genomic sequence, as well as the ca^{+2} dependent phosphocholine binding character found in CRPs [40]. The molecular mass of limuli was refined to 300 kDa. Its crystallographic structure was proved to have 12 subunits of each having 25 kDa mass arranged in hexameric arrangement [41]. They are organized in hexagonal subunits encoded by 3 different introns less genomes. Despite changes in quaternary structure, comparative examinations of the limulin structure based on the 3D structure of SAP component show significant support for a largely preserved 3 phase fold. In the horseshoe crab, it was discovered that limulin, together with the C3-macroglobulin, engages in a complement like hemolytic activity, with both molecules being necessary for optimum function. There have also been reports of other lectins superfamily with CRP-like behavior [40,41]

Rat: -The rat CRP was further characterized by the discovery of single Chain oligosaccharide connected to Asn128 [42]. This chain is not involved in dimer or oligomer formation, PC binding. The 3D crystallographic arrangement of rat CRP was studied using preliminary X-ray analysis.

American & Syrian Hamster; -The Syrian hamster SAP is known as serum female protein. This PTX is distinctive in that both sex hormones and cytokines influence the gene's Pre translational expression [43] . The SAP component gene was displayed to be physically identical to pentraxin genes found in numerous animals. Androgenic hormones decrease its production at a early stage, owing to a change in transcriptional rate, at least in part. To make a comparison, the structure of SAP in case of Armenian hamster protein was studied. Although the specific regions of both the Armenian and Syrian hamsters' SAP component are very similar, there are significant variances in the putative enhancer locations. Therefore, a rapid SAP response can trigger a noticeable absence of change in liver levels of reactions leading to synthesis of either SAP or CRP after endotoxin stimulation [44]. Reexamination of the properties of SAP component revealed a biomolecule composed of 5 glycosylated folds of 25 655 MW (each having one disulfide bridge inside the chain structure) that combine to form decamers in the presence of calcium. At physiological ion concentration, the other pentraxins do not show this shift [44,45].

African clawed frog In case of amphibians *(Xenopus laevis)*, the pentraxin component has been identified as Containing 222 amino-acids with a 16-residue signal peptide chain having mass of 24 KDa [46]. This is detectable in very low concentration (1.0 gg ml-l) in serum. Unlike human PTX, Xenopus PTX is not activated by IL-6 and heat shock [47]. Sensitive regions seen in the upstream promoter regions of other CRP alleles. Xenopus PTX shares 39-45 % sequence homology with PTX from higher vertebrates and 24 % with the Crab, regarding many areas exhibiting substantially higher homology (up 57 Instinctive resistances to 89 %) [48]. The cloned gene is made of 3 exon and 2 intron sequence. The initial intron is absent from other PTX sequences which makes it different. In addition, a new member of the pentraxin called as long neuronal pentraxin-1(XLPXN-1) was discovered, that is roughly double the size of a CRP isoform has been discovered. This PTX is similar with PTX3 gene in humans [49].

Guinea Pig: -The structural shapes and function of the PTX genomes were identified in the guinea pig species. The SAP component presence in pig was explained before. In this species, neither pentraxin performs as a most important severe stage reactant. Genomic genetic copy of short PTX were separated and sequenced, and the gene and obtained protein sequences matched with other mammal species [50]. Both genes have associations with standard of the PTX genes of other varieties, but some variations were described in the zones that establish the capability of the pentraxin gene to be stimulated in the course of critical infection. These findings validate the general preservation of genomic association of the pentraxins in mammals, and also reinforce an obvious loss of inherited components that might recognize areas that are important during the severe time countered response [51].

Zebra Fish :-I was interested in the previously unreported regulation of the expression of several zebrafish CRPs in response to the skin. On the one hand, neither significant lymphoid tissue nor APR-related tissue are thought to exist in the skin. However, one of the rhabdovirus's preferred entry points into fish is through the skin, which explains the fish's quick and noticeable response. Diffuse mucosa-associated lymphoid tissue (MALT), a form of secondary lymphoid tissue that is not only endowed with substantial innate immune activity but also with pertinent adaptive immunity features, is also present in the skin, which is the greatest immunologically active organ in fish [52]. There is also conformational change of structure found in case of zebra fish because it forms a trimeric structure. It forms a dare PTX structure whose sequence identity was low with comparison to other species. Although there is a less sequence identity between zebra fish and human PTX but their functional motifs, and binding to the C1q molecule of complement system was conserved throughout the evolution. The reason why the zebra fish forms the trimeric structure is the distance between the interface of different subunit and residues which forms a hydrophobic core.

4. DISCUSSION

4.1 Why Zebra Fish pentraxins are trimeric?

Zebra Fish shows a Dare-PTX due to its unique configuration. The subunits are arranged in a planner symmetry to form a hydrophobic core unit which is not ion case of human and limulus [53]. Although the protomers have similarity with the human and limulus protomers but their diversity in arrangement between the protomers gives a unique idea about the species. In the case of Zebra Fish Two protomers contacting both faces of the trimeric unit forms a hydrophobic pore which makes it distant from others.

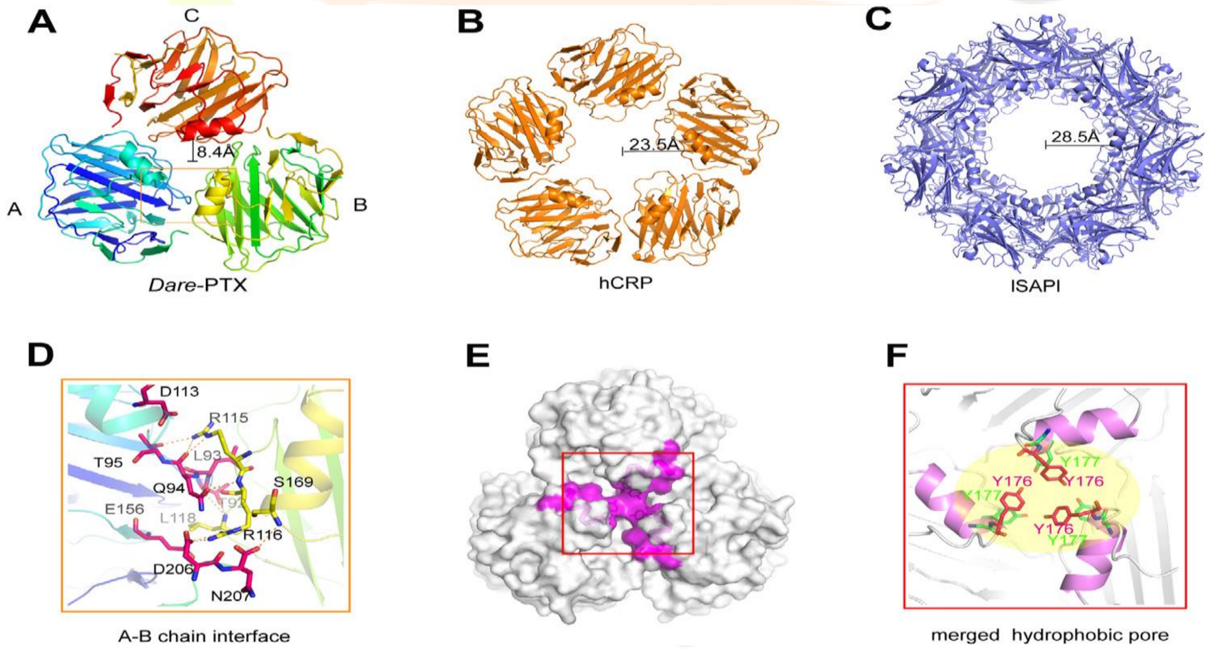


Fig.13 *(A) Unique structure of Zebra fish pentraxins forming a trimeric structure caused by a formation of hydrophobic pore region. (B) Structure of human pentraxin having pentameric symmetry on a planner surface having diameter 23.5 A⁰. (C) Structure of heptameric limulus showing the protomers are arranged to form the stabilized heptameric structure. (D) Interaction between the two sides of Dare-PTX where one side of A chain and another side B face forms the hydrophobic pore unit. (E) 3D view of hydrophobic core. (F) Formation of Merged hydrophobic pore between Tyr 177 and Tyr 176 shown in sticks.*

4.2 ***Pentraxins and Complement System***

CRP starts the traditional acute cascade by attaching directly to C1q. This is the initial element of the classic route. Similarly to IgG, each CRP unit has one C1q binding domain. The C1 activation requires at least two CRP molecules [54]. Till the date crystallographic structure for the CRP-C1q molecule interaction has not been found. CRP was first discovered to bind with the cross-linked stalk of the C1q domain. Single class of C1q molecule cooperates with the core unit of CRP on the A site contacting the binding site, according to a molecular model. This hypothesis is based on investigations of the side specific binding of C1q using site-directed mutagenesis. SAP binds to same cascade and triggers the classical pathway. CRP complement activation appears to be quite similarity to the action of IgM/IgG at first glance [55]. Furthermore, a closer look conforms that CRP synthesis is not responsible for the formation of the membrane assault complex as efficiently.

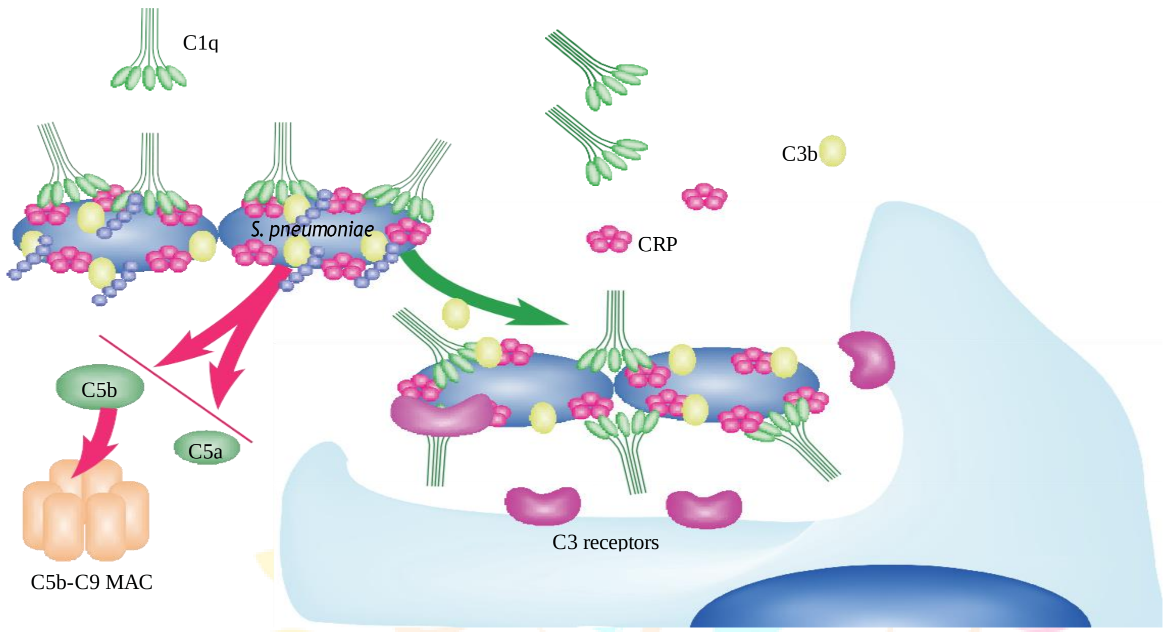


Fig.14 *CRP complexes' activation of the complement cascade. The Gram-positive bacterium S. pneumoniae, which expresses repetitive ligands like PC attachment with CRP. Single C1q molecule interacts with a single CRP pentamer. C1 activation, along with proteolytic cleavage, and the activation of a C3 convertase enzyme occur because of C1q's interaction with many CRP molecules.*

4.3 Pentraxins in infection control

The first hint that CRP might help prevent infection came from CRP attaching to S. pneumoniae. The specific integration a of short PTX for *S. pneumoniae* was initially observed by Kind mark's team. It was later shown that CRP might defend mice against tentative *S. pneumoniae* contamination and that a major portion of this benefit was mediated by complement activation. The human CRP was observed through a transgenic, shield from pneumococcal contamination was also found [56].

Salmonella typhimurium infection was also prevented in CRP mice. *S. pneumoniae*, *Hemophilus influenza*, and other infective organisms contain the surface-expressed PC which are recognized by CRP as pathogens. The PC attachment pocket is required for defense from the *S. pneumoniae*, according to mutagenesis experiments [57]. According to Weiser et al., the action of PC on *influenzae* virus permitted CRP attachment and complement activation. Additionally, the fact was established that CRP could be present in these secretions and that it is uttered in the respirational region [56,57]. Thus, CRP may behave as a barrier, similar to IgA, and may directly defend against respiratory tract infections by activating complement.

In a Sudanese community, a link among inherently defined amounts of CRP utterance and malaria disease was studied. The CRP gene's upstream polymorphism, which is seen to affect CRP concentrations, was studied in this work. Baseline CRP expression is most abundant in the A variant. In this study, the CRP 286 gene locus was genotyped in a group of 192 Sudanese contributors who were monitored for malaria infection over a nine-year period. CRP nucleotides like A, C, and T were most common, with prevalence rates of 21%, 52%, and 27%, respectively [58]. Each person's overall number of malaria incidents throughout the class of the research served as an indicator of their vulnerability to the disease. Each person's total quantity of malaria incidents during the study served as an indicator of their vulnerability to the disease. In contrast to the CRP genetic constitution, the A-allele remained substantially higher figure in case of malaria incidences having superior organism amounts ($P = 0.007$). A-allele carriers made up 18% of donors who were not infected by malaria [59].

4.4 CRP as an acute phase reactant

The contact of CRP with neutrophils has been investigated around several centuries. The first specified action of short PTX on neutrophils was its ability to counteract both bacterial (Gram + & Gram -) pathogens [59]. Researchers proved that CRP and defence mechanism could provoke phagocytosis of red blood cell glazed along with the polyose of *S. pneumoniae* [60].

Additionally, it was confirmed that CRP restrains neutrophils, specifically neutrophil activation and phagocytosis. For instance, Webster's team discovered that CRP might prevent neutrophil activation both in vitro and in vivo. According to one theory, p38 MAP kinase inhibition was responsible for these results. Similar results were obtained from Zhong etal investigation into CRP's impact on neutrophil chemotaxis. "He discovered that IL-8 and FMLP (formyl-methionyl-leucyl-pheylalanine) chemotactic signal reduced neutrophil chemotaxis in response to CRP" [61]. IgG-induced chemiluminescence was found to be enhanced by aggregated

CRP, according to Zeller and Sullivan. Antibodies to FcRII/III significantly suppressed this action. But the main exemption here is that these experiments are performed before, and human and mouse receptors are considered as the blocking pathways for different antibodies [62].

The Proteins associated with complement activation show a specific response inside the body. The SAP component was continuously expressed at the time of infection whole normal amount inside the male blood is 40µg/mL [63]. The name of protein also suggests that its physical attachment with amyloid fibers in different forms. The main function of these proteins are quite unclear inside the body. The pentraxins are resistant to extreme environment due their tightly paced structure. But it was clear that they have also the inflammatory response inside the body with responses to specific cytokines like IL-6 &IL-10 [64]. When some infections are detected inside the body these proteins are immediately synthesized inside the kidney to activate the complement system. The specific binding affinity towards the antigenic ligands helps to attach and trigger immune responses. Also, these proteins have functions like Phagocytosis, Neutrophil activation, Cell removal due to apoptosis and necrosis represented in ***Fig.15***.

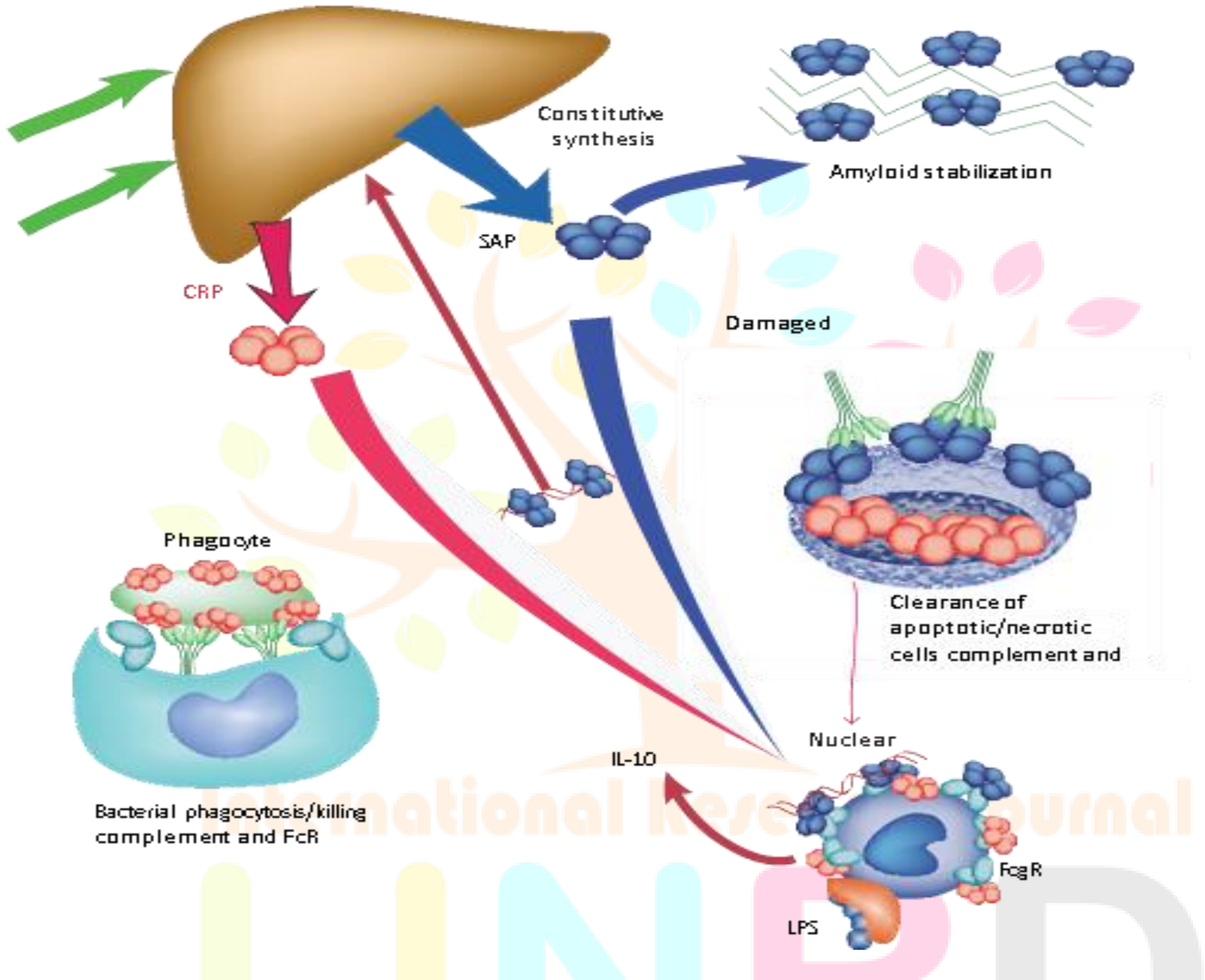


Fig.15 *Acute phase response of both CRP and SAP component inside human body. In response to specific foreign materials like pathogens. Both the proteins are synthesized inside liver in response to specific cytokines. They have specific response towards the host defense in order to eradicate the antigens by synthesis of complement activation complex and amyloid stabilization. The side specific ligand binding site for both the proteins shows similar action to eliminate the cells due apoptosis and necrosis. In response to cytokines like IL-10 it binds to nuclear antigens containing Lipopolysaccharides. This process also acts similar manner in the phagocytosis and Neutrophil activation [65].*

4.5 Clinical Application of CRP

There are two approaches to use CRP values in therapeutic settings. A positive reading was used for many years as a sign of inflammation or infection because the original methods are used to quantify CRP in the human body which is insensitive. CRP is a highly effective indicator of the early inflammatory response and is frequently meant for rheumatologic and other illness diagnosis as well as prognosis. In the intense phase response, CRP concentrations range from 10 g/mL to 500 g/mL [66]. CRP is a component of the Disease Activity Score 28 and is frequently used to assess disease activity in rheumatoid arthritis. Like this, assessing CRP levels is useful for keeping track of the progression of certain vasculitis diseases. Some assays were developed 20 years ago to measure relative CRP amount inside the human body. But the main difficulty is to detect a lower amount of CRP inside the body.

5. CONCLUSION

A group of evolutionarily conserved proteins known as pentraxins which might be traced back to the earliest organisms. They have developed via combining through the ancestor complement system, the cell receptors, and the innate and adaptive immune systems. Their most well-known function is innate immune response, but they remained also crucial sample-identification biomolecules for alterations in self-antigens. These group of proteins has been examined a long years ago, but their relative functions are quite unclear at present time also.

While SAP is expressed by default, inflammatory activities enhance CRP generation. Although CRP has been the subject of considerably more research, both pentraxins play a part in host defence by activating the Inflammation complement. Additionally, both PTXs attach to injured cells and nuclear cellular elements to enable their secure elimination in a nonimmunogenic way. CRP decreases inflammation, while in the case of SAP, this inhibits tissue thickening. The pentraxins, in general, offer a monitoring route to manage the seditious reaction to tissue damage. This group of protein can be largely used as a biomarker for different diseases.

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7. REFERNCES

1. Kaplan R, Li SSL, Kehoe JM. Molecular Characterization of Limulin, a Sialic Acid Binding Lectin from the Hemolymph of the Horseshoe Crab, Limulus polyphemus. *Biochemistry*. 1977;16(19):4297-4303. doi:10.1021/bi00638a026
2. Clos TW du. Pentraxins: Structure, Function, and Role in Inflammation. *ISRN Inflammation*. 2013; 2013:1-22. doi:10.1155/2013/379040
3. Vizzini A, Dumas F, di Falco F, Arizza V. Evolutionary and transcriptional analyses of a pentraxin-like component family involved in the LPS inflammatory response of Ciona robusta. *Fish & Shellfish Immunology*. 2021;111:94-101. doi:10.1016/J.FSI.2021.01.014
4. Pepys MB. The pentraxins 1975-2018: Serendipity, diagnostics and drugs. *Frontiers in Immunology*. 2018;9(OCT):2382. doi:10.3389/FIMMU.2018.02382/BIBTEX
5. Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T, Shelton E. Characterization of C reactive protein and the complement subcomponent C1t at homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc Natl Acad Sci U S A*. 1977;74(2):739-743. doi:10.1073/PNAS.74.2.739
6. Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. *Structure*. 1994;2(11):1017-1027. doi:10.1016/S0969-2126(94)00105-7
7. Pepys MB, Rademacher TW, Amatayakul-Chantler S, et al. Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. *Proc Natl Acad Sci U S A*. 1994;91(12):5602-5606. doi:10.1073/PNAS.91.12.5602
8. Emsley J, White HE, O'Hara BP, et al. Structure of pentameric human serum amyloid P component. *Nature 1994 367:6461*. 1994;367(6461):338-345. doi:10.1038/367338a0
9. Koussih L, Atoui S, Tliba O, Gounni AS. New Insights on the Role of pentraxin-3 in Allergic Asthma. *Frontiers in allergy*. 2021;2. doi:10.3389/FALGY.2021.678023
8. 10. Gewurz H, Zhang XH, Lint TF. Structure and function of the pentraxins. *Curr Opin Immunol*. 1995;7(1):54-64. doi:10.1016/0952-7915(95)80029-8
11. Breviario F, D'Aniello EM, Golay J, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *Journal of Biological Chemistry*. 1992;267(31):22190-22197. doi:10.1016/S0021-9258(18)41653-5
12. Goodman AR, Cardozo T, Abagyan R, Altmeyer A, Wisniewski HG, Vilček J. Long pentraxins: an emerging group of proteins with diverse functions. *Cytokine & Growth Factor Reviews*. 1996;7(2):191-202. doi:10.1016/1359-6101(96)00019-6
13. Noland TD, Friday BB, Maulit MT, Gerton GL. The sperm acrosomal matrix contains a novel member of the pentaxin family of calcium-dependent binding proteins. *Journal of Biological Chemistry*. 1994;269(51):32607-32614. doi:10.1016/S0021-9258(18)31677-6
14. Gilgès d, Vinit ma, Callebaut i, et al. Polydom: a secreted protein with pentraxin, complement control protein, epidermal growth factor and von Willebrand factor A domains. *Biochemical Journal*. 2000;352(1):49-59. doi:10.1042/BJ3520049
15. Bjarnadóttir TK, Fredriksson R, Höglund PJ, Gloriam DE, Lagerström MC, Schiöth HB. The human and mouse repertoire of the adhesion family of G-protein-coupled receptors. *Genomics*. 2004;84(1):23-33. doi:10.1016/J.YGENO.2003.12.004
16. Torre YM de la, Fabbri M, Jaillon S, et al. Evolution of the Pentraxin Family: The New Entry PTX4. *The Journal of Immunology*. 2010;184(9):5055-5064. doi:10.4049/JIMMUNOL.0901672
17. Vizzini A, Dumas F, di Falco F, Arizza V. Evolutionary and transcriptional analyses of a pentraxin-like component family involved in the LPS inflammatory response of Ciona robusta. *Fish & Shellfish Immunology*. 2021;111:94-101. doi:10.1016/J.FSI.2021.01.014
18. Li Y, Li J, Yan X, et al. Broad distribution, high diversity and ancient origin of the ApeC-containing proteins. *Molecular Phylogenetics and Evolution*. 2021;155:107009. doi:10.1016/J.YMPEV.2020.107009

19. Balhara J, Koussih L, Zhang J, Gounni AS. Pentraxin 3: An immuno-regulator in the lungs. *Frontiers in Immunology*. 2013;4(MAY):127. doi:10.3389/FIMMU.2013.00127/BIBTEX
20. Kim Y, Park JS, Park HJ, et al. Pentraxin 3 Modulates the Inflammatory Response in Human Dental Pulp Cells. *Journal of Endodontics*. 2018;44(12):1826-1831. doi:10.1016/J.JOEN.2018.08.003
21. Ponticelli C, Campise MR. The inflammatory state is a risk factor for cardiovascular disease and graft fibrosis in kidney transplantation. *Kidney International*. 2021;100(3):536-545. doi:10.1016/J.KINT.2021.04.016
22. Liu B, Ai P, Zheng D, et al. Cerebrospinal fluid pentraxin 3 and CD40 ligand in anti-N-menthyl-d-aspartate receptor encephalitis. *Journal of Neuroimmunology*. 2018;315:40-44. doi:10.1016/J.JNEUROIM.2017.11.016
23. Fan WC, Huang CC, Yang YY, et al. Serum pentraxin-3 and tumor necrosis factor-like weak inducer of apoptosis (TWEAK) predict severity of infections in acute decompensated cirrhotic patients. *Journal of Microbiology, Immunology and Infection*. 2017;50(6):905-914. doi:10.1016/J.JMII.2015.12.006
24. Pertsemlidis A, Fondon JW. Having a BLAST with bioinformatics (and avoiding BLAST phemy). *Genome Biology*. 2001;2(10):1-10. doi:10.1186/GB-2001-2-10-REVIEWS2002/FIGURES/4
25. Kannan L, Wheeler WC. Maximum Parsimony on Phylogenetic networks. *Algorithms Mol Biol*. 2012;7(1). doi:10.1186/1748-7188-7-9
26. AlphaFold Protein Structure Database. Accessed June 30, 2022. https://alphafold.ebi.ac.uk/
27. Tables Tutorial: Visualize Molecules like AlphaFold-ed Proteins – Weights & Biases. Accessed June 30, 2022. https://wandb.ai/wandb/examples/reports/Tables-Tutorial-Visualize-Molecules-like-AlphaFold-ed-Proteins--Vmlldzo4ODc2ODY
28. Practical Pymol for Beginners - PyMOLWiki. Accessed July 16, 2022. https://pymolwiki.org/index.php/Practical_Pymol_for_Beginners
29. Introduction to PyMOL. Published online 2009.
30. PDBsum home page. Accessed July 16, 2022. http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html
31. Martinez de la Torre Y, Fabbri M, Jaillon S, et al. Evolution of the pentraxin family: the new entry PTX4. *J Immunol*. 2010;184(9):5055-5064. doi:10.4049/JIMMUNOL.0901672
32. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. 2005;23:337-366. doi:10.1146/ANNUREV.IMMUNOL.23.021704.115756
33. Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T, Shelton E. Characterization of C reactive protein and the complement subcomponent C1t at homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc Natl Acad Sci U S A*. 1977;74(2):739-743. doi:10.1073/PNAS.74.2.739
34. Hutchinson WL, Hohenester E, Pepys MB. Human serum amyloid P component is a single uncomplexed pentamer in whole serum. *Mol Med*. 2000;6(6):482-493. doi:10.1007/BF03401789/FIGURES/5
35. Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. *Structure*. 1994;2(11):1017-1027. doi:10.1016/S0969-2126(94)00105-7
36. Pepys MB, Rademacher TW, Amatayakul-Chantler S, et al. Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. *Proc Natl Acad Sci U S A*. 1994;91(12):5602-5606. doi:10.1073/PNAS.91.12.5602
37. Shrive AK, Cheetham GMT, Holden D, et al. Three dimensional structure of human C-reactive protein. *Nature Structural Biology 1996 3:4*. 1996;3(4):346-354. doi:10.1038/nsb0496-346
38. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure*. 1999;7(2):169-177. doi:10.1016/S0969-2126(99)80023-9
39. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today*. 1994;15(2):81-88. doi:10.1016/0167-5699(94)90138-4
40. liu t -y, Minetti cas, Fortes-dias cl, Liu t, Lin l, lin y. C-reactive proteins, limunectin, lipopolysaccharide-binding protein, and coagulin. Molecules with lectin and agglutinin activities from Limulus polyphemus. *Ann N Y Acad Sci*. 1994;712(1):146-154. doi:10.1111/J.1749-6632.1994.TB33569.X
41. Vasta GR, Marchalonis JJ, Kohler H. Invertebrate recognition protein cross-reacts with an immunoglobulin idiotype. *Journal of Experimental Medicine*. 1984;159(4):1270-1276. doi:10.1084/jem.159.4.1270
42. Chan AL, Morris HR, Panico M, et al. A novel sialylated N-acetylgalactosamine-containing oligosaccharide is the major complex-type structure present in bowes melanoma tissue plasminogen activator. *Glycobiology*. 1991;1(2):173-185. doi:10.1093/glycob/1.2.173
43. Rudnick cm, Dowton sb. Serum Amyloid-P Component of the Armenian Hamster: Gene Structure and Comparison with Structure and Expression of the SAP Gene from Syrian Hamster. *Scandinavian Journal of Immunology*. 1993;38(5):445-450. doi:10.1111/j.1365-3083.1993.tb02586.x
44. Coe JE, Ross MJ. Electrophoretic polymorphism of a hamster pentraxin, female protein (Amyloid P Component). *Scandinavian Journal of Immunology*. 1997;46(2):180-186. doi:10.1046/j.1365-3083.1997.d01-109.x
45. Coe JE. female protein of the the syrian hamster: a homolog of c-reactive protein. *Ann N Y Acad Sci*. 1982;389(1):299-307. doi:10.1111/j.1749-6632.1982.tb22145.x
46. Viegas CSB, Pinto JP, Conceição N, Simes DC, Cancela ML. Cloning and characterization of the cDNA and gene encoding Xenopus laevis osteocalcin. *Gene*. 2002;289(1-2):97-107. doi:10.1016/S0378-1119(02)00480-8
47. Murphy TM, Baum LL, Beaman KD. Extrahepatic transcription of human C-reactive protein. *Journal of Experimental Medicine*. 1991;173(2):495-498. doi:10.1084/jem.173.2.495
48. Maéno M, Ying X, Wood TI, Ong RC, Hsiang-fu K. Cloning and expression of cDNA encoding Xenopus laevis bone morphogenetic protein-1 during early embryonic development. *Gene*. 1993;134(2):257-261. doi:10.1016/0378-1119(93)90103-A

49. Isolation and characterization of C-reactive protein (CRP) cDNA and genomic DNA from Xenopus laevis. A species representing an intermediate stage in CRP evolution - PubMed. Accessed July 18, 2022. https://pubmed.ncbi.nlm.nih.gov/8454653/
50. Morimatsu M, Sakai H, Yoshimatsu K, et al. Isolation and characterization of C-reactive protein and serum amyloid P component from bovine serum. *Nippon juigaku zasshi The Japanese journal of veterinary science*. 1989;51(4):723-732. doi:10.1292/jvms1939.51.723
51. Ptx3 pentraxin 3 [Cavia porcellus (domestic guinea pig)] - Gene - NCBI. Accessed July 18, 2022. https://www.ncbi.nlm.nih.gov/gene/100726587
52. Patrikainen MS, Tolvanen MEE, Aspatwar A, et al. Identification and characterization of a novel zebrafish (Danio rerio) pentraxin-carbonic anhydrase. *PeerJ*. 2017;5(12). doi:10.7717/PEERJ.4128
53. Patrikainen MS, Tolvanen MEE, Aspatwar A, et al. Identification and characterization of a novel zebrafish (Danio rerio) pentraxin-carbonic anhydrase. *PeerJ*. 2017;2017(12). doi:10.7717/PEERJ.4128/
54. Du Clos: Complement and complement deficiencies - Google Scholar. Accessed July 18, 2022. https://scholar.google.com/scholar_lookup?title=Complement%20and%20complement%20deficiencies&author=T.%20W.%20Du%20Clos%20&author=C.%20Mold&publication_year=2013
55. Lin PM, Wright JR. Surfactant protein A binds to IgG and enhances phagocytosis of IgG-opsonized erythrocytes. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2006;291(6). doi:10.1152/AJPLUNG.00188.2006
56. Kindmark CO. In vitro binding of human C-reactive protein by some pathogenic bacteria and zymosan. *Clinical and Experimental Immunology*. 1972;11(2):283. Accessed July 18, 2022. /pmc/articles/PMC1553622/?report=abstract
57. Mold C, Rodriguez W, Rodic-Polic B, Clos TW du. C-Reactive Protein Mediates Protection from Lipopolysaccharide Through Interactions With FcγR. *The Journal of Immunology*. 2002;169(12):7019-7025. doi:10.4049/JIMMUNOL.169.12.7019
58. Schuldt K, Esser C, Evans J, et al. FCGR2A functional genetic variant associated with susceptibility to severe malarial anaemia in Ghanaian children. *Journal of Medical Genetics*. 2010;47(7):471-475. doi:10.1136/JMG.2009.073643
59. Naik P, Voller A. Serum C-reactive protein levels and falciparum malaria. *Trans R Soc Trop Med Hyg*. 1984;78(6):812-813. doi:10.1016/0035-9203(84)90027-0
60. Kindmark CO. Stimulating effect of C-reactive protein on phagocytosis of various species of pathogenic bacteria. *Clinical and Experimental Immunology*. 1971;8(6):941. Accessed July 18, 2022. /pmc/articles/PMC1713038/?report=abstract
61. Hokama y, coleman mk, riley rf. in vitro effects of c-reactive protein on phagocytosis. *Journal of Bacteriology*. 1962;83:1017-1024. doi:10.1128/JB.83.5.1017-1024.1962
62. Lu J, Marjon KD, Marnell LL, et al. Recognition and functional activation of the human IgA receptor (FcαRI) by C-reactive protein. *Proc Natl Acad Sci U S A*. 2011;108(12):4974-4979. doi:10.1073/PNAS.1018369108/SUPPL_FILE/PNAS.201018369SI.PDF
63. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration That C-Reactive Protein Decreases eNOS Expression and Bioactivity in Human Aortic Endothelial Cells. *Circulation*. 2002;106(12):1439-1441. doi:10.1161/01.CIR.0000033116.22237.F9
64. Haudek SB, Trial JA, Xia Y, Gupta D, Pilling D, Entman ML. Fc receptor engagement mediates differentiation of cardiac fibroblast precursor cells. *Proc Natl Acad Sci U S A*. 2008;105(29):10179-10184. doi:10.1073/PNAS.0804910105/ASSET/A52E9537-2050-45D5-BE45-90F01246B997/ASSETS/GRAPHIC/ZPQ9990841000006.JPEG
65. du Clos TW. Pentraxins: Structure, Function, and Role in Inflammation. *ISRN Inflammation*. 2013;2013:1-22. doi:10.1155/2013/379040
66. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *The Journal of Clinical Investigation*. 2003;111(12):1805-1812. doi:10.1172/JCI18921